Stability of infectious HIV in clinical samples and isolation from small volumes of whole blood

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Abstract

Aims—To evaluate the stability of infectious HIV in clinical samples and the efficiency of isolating it from small volumes of whole blood.

Methods—Titres of infectious HIV were measured in peripheral blood mononuclear cells and plasma 2, 24, and 48 hours after peripheral blood had been collected from 10 HIV positive adult volunteers. Volumes of whole blood (1 ml to 5 μl), collected from a further five volunteers, were used to determine the minimum volume from which HIV could be isolated. Infectious HIV was isolated by co-culture with phytohaemagglutinin stimulated umbilical cord mononuclear cells.

Results—Geometric mean titres of infectious HIV seemed to be more stable in peripheral blood mononuclear cells than in plasma. HIV was recovered from all 10 peripheral blood mononuclear cell samples during the 48 hours after sample collection, but from only four plasma samples. HIV could occasionally be isolated from 5 μl of whole blood and reliably from 200 μl.

Conclusions—HIV can be isolated from peripheral blood mononuclear cells and plasma for up to 48 hours after sample collection. Isolation of HIV from small volumes of whole blood has applications for the diagnosis and management, of HIV positive children.

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There is little information on the stability of infectious HIV in clinical material or on the efficiency of isolating HIV from small volumes of blood. Such information is relevant for studies in which processing may be delayed, as in worldwide multi-centre studies when samples may be in transit for some time. Titres of infectious HIV could decrease considerably during transit thus jeopardising studies in which infectivity titres are used to assess disease progression or for monitoring the efficacy of antiviral chemotherapy. Rapid diagnosis of HIV infection in infants born to infected mothers is extremely important if appropriate support and management, including prophylaxis of opportunistic infections and administration of antiviral chemotherapy, is to be initiated at an early age. Serological methods are of limited value in children younger than 18 months because of the persistence of maternal antibodies. Most infected infants, however, can now be identified by the age of 2 months by virus isolation and the polymerase chain reaction. Rapid diagnosis may, however, be limited by the sample volume, because in addition to virological assays, immunological, haematological, and biochemical assays are often required. Determining whether quantitative isolation of HIV can be reliably conducted with very small volumes of whole blood, therefore, has an important application in terms of paediatric diagnosis and management.

We are currently conducting an international multi-centre study on vertical transmission of HIV. Before starting this study we had to determine the stability of infectious HIV in peripheral blood mononuclear cells (PBMCs) and plasma as well as the minimum volume of whole blood from which HIV could be isolated, virus isolation being one of the tests to be used for paediatric diagnosis. This paper presents the results of these investigations.

Methods

Fifteen HIV seropositive adult volunteers were recruited from patients attending the Department of Genitourinary Medicine, St Thomas’s Hospital, London. Informed consent was obtained from all volunteers. Seven were asymptomatic Centers for Disease Control (CDC stage IV) and eight were asymptomatic (CDC stage II). The mean CD4 number in the symptomatic volunteers was 0·07 × 10⁹/l (range 0·04–0·18) and in the asymptomatic volunteers it was 0·37 × 10⁹/l (range 0·11–0·56). None of the volunteers was being treated with zidovudine at the time of sample collection. Ten of the 15 volunteers provided 15 ml of blood anticoagulated with heparin. This was kept at room temperature and 5 ml aliquots were removed and PBMCs and plasma processed within two hours of collection and after 24 and 48 hours. To evaluate the efficiency of isolating HIV from small volumes of whole blood, the remaining five volunteers provided 5 ml of blood anticoagulated with heparin from which 1 ml to 5 μl were co-cultured as whole blood within two hours of collection.

HIV was quantified in PBMC, plasma, and whole blood using previously described methods. Briefly, serial numbers of PBMCs, and dilutions of plasma and whole
blood, were co-cultured with phytohaemagglutinin (PHA) stimulated cord blood mononuclear cells from at least two HIV seronegative umbilical cord bloods. The three 5 ml samples from the same individual, processed at different times, were always co-cultured with the same batch of PHA stimulated cord cells. Cultures were maintained for 28 days and supernatant fluids tested for p24 antigen using enzyme immunoassay (Abbott Diagnostics Division, North Illinois, USA); positive cultures were confirmed by neutralisation. Titres of HIV were expressed as a tissue culture infective dose (TCID) per 10^6 PBMC, per ml of plasma or per ml of whole blood.

Infectivity titres were log transformed and geometric mean titres (GMT) calculated taking titres of < 1 and < 0·5 as 1·0 and 0·5, respectively. To evaluate changes in infectivity titre during the 48 hour period 95% confidence intervals were calculated for the geometric mean ratio change between two and 24 hours and two and 48 hours, a ratio of 1·0 indicating no change in titre.

Results

HIV was isolated from all 10 PBMC samples during the 48 hour period (table 1). The proportion of positive isolations did, however, vary at two hours (seven out of 10), 24 hours (nine out of 10), and 48 hours (nine out of nine) after sample collection. Only one volunteer (case 3) had the same titre of infectious HIV in PBMCs during the 48 hour period. In three cases (cases 4,7 and 10) HIV was not detected in samples processed at two hours and in one of these volunteers (case 4) virus was only detected in PBMCs processed 48 hours after collection of blood. One volunteer (case 1) showed a 100-fold increase in titre and six (cases 2,5,6,7,8,9,10) a 10-fold decrease in titre during the 48 hour period. The GMT of HIV in PBMCs processed two, 24, and 48 hours after sample collection was 6·3, 15·8, and 6·5 TCID/10^6 PBMCs, respectively (table 1). The 95% confidence interval for the geometric mean ratio change between two and 24 hours was 0·36 to 17·3 (p > 0·05) and between two and 48 hours 0·1 to 10·4 (p > 0·05).

Table 1 Titres of HIV in PBMCs and plasma kept at room temperature for up to 48 hours

<table>
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<tr>
<th>Volunteer</th>
<th>CDC stage</th>
<th>PBMC*</th>
<th>Plasma**</th>
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<th>Plasma</th>
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<tr>
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<td>4·7</td>
<td>15·8</td>
<td>1·9</td>
<td>6·5</td>
<td>1·4</td>
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ND Not done.
*TCID/10^6 PBMCs.
**TCID/ml plasma.

HIV was isolated from the plasma of four volunteers (cases 5,8,9,10) during the 48 hour period (table 1) and all were CDC stage IV with a mean CD4 count of 0·05 × 10^4/1. All four showed a decrease in infectivity titre during the 48 hour period and in two cases (cases 9 and 10) infectious virus was no longer detectable 48 hours after sample collection. The GMT in plasma processed two, 24, and 48 hours after sample collection was 4·7, 1·9, and 1·4 TCID/ml of plasma, respectively. The 95% confidence interval for the geometric mean ratio change between two and 24 hours was 0·13 to 1·2 (p > 0·05) and between two and 48 hours 0·07 to 0·97 (p < 0·05).

HIV was isolated from all five whole blood samples which were processed within two hours of collection (table 2). Infectious virus was isolated from between 5 µl and 200 µl of whole blood. The GMT was 22 TCID/ml with a range of 5–200 TCID/ml of blood.

Discussion

We are aware of only one other study in which the stability of HIV in clinical samples has been evaluated. This is perhaps surprising but probably reflects the fact that such studies are very labour intensive; our study, involving 10 patients, required 360 co-cultivations. The recent study by Moudgil and Daar, unlike our study, in which HIV titres were measured in both PBMCs and plasma, only determined infectivity titres in plasma. They showed that complete decay of infectious HIV in the plasma of seven patients could take more than seven days and titres were found to be more stable when plasma was stored at 4°C. A study assessing the stability of HIV in culture supernatant fluids showed the presence of infectious virus after 15 days at room temperature, but the titre of infectious virus detected at this time was not given. A high titre (10^7 TCID/ml) laboratory strain was also used and results may not be applicable to clinical samples where levels of virus are usually at least 1000-fold lower. Although studies have been reported in which HIV has been isolated from the blood of infected cadavers for up to five days after death, they did not indicate whether a significant decrease in viral titre occurred during this period. A retrospective study of transfusion transmitted HIV showed a lower infection rate among patients transfused with infected blood that had been stored for 22 days than among persons transfused with infected blood stored for less than eight days,
thus indicating a loss of infectious virus with storage.

Our study shows that infectious HIV can be recovered from PBMCs and some plasma samples for up to 48 hours after sample collection. Virus was recovered more frequently and also appeared to be more stable in PBMCs, however, than in plasma, as indicated by a similar GMT in PBMCs but not in plasma two hours and 48 hours after sample collection. This could in part reflect a more rapid loss of infectivity when HIV is extracellular. Failure to demonstrate a significant difference in the titre of virus in PBMCs over the 48 hour period could also reflect the small number of samples tested particularly as in some individual cases infectivity titres varied considerably in both PBMCs and plasma. HIV was isolated from the PBMCs of all volunteers regardless of their disease stage whereas isolation from plasma was associated with more advanced disease, as shown by other workers.19 We have already shown that although HIV can be isolated from the PBMCs of all infected volunteers, titres are highest among those with more advanced disease.3

Although the mean infectivity titre was higher in PBMCs processed 24 hours after collection than in samples processed within two hours, this difference was not significant and may have simply reflected the small number of samples tested. In contrast, there was a significant decrease in the titre of virus in plasma during the 48 hour period. On the basis of our results we consider that blood samples are acceptable for quantitative isolation of HIV if they have been in transit for no longer than 24 hours at ambient temperature. It should be appreciated, however, that there may be some loss of infectious virus in plasma during this time and it may therefore be more appropriate to transport plasma samples at 4°C as suggested by Moudgil and Daar.4

A question frequently asked by paediatricians is the minimum volume of blood required for diagnosis of paediatric HIV infection. Diagnosis and clinical management is likely to depend on the results obtained from a variety of tests but this often requires a considerable volume of infant blood. Tests which can utilise very small volumes of blood, such as the polymerase chain reaction, are therefore ideally suited for paediatric diagnosis.3 This study shows that HIV diagnosis can also be made by quantitative virus isolation using very small volumes of whole blood. We isolated HIV from all five whole blood cultures—in two cases from as little as 5 μl and 20 μl of blood. Advantages of whole blood culture are that it obviates the need for gradient purification of PBMCs and cultures can be established quickly and with small volumes. These are features that make the assay ideally suited for paediatric diagnosis and management.

We have already used the technique to assess eight infants born to HIV seropositive mothers and have diagnosed infection in two. In one case HIV was isolated from 200 μl of cord blood eight days after whole blood cultures were established. In the second case the first specimen was not obtained until the infant was aged 3 months and HIV was isolated nine days after whole blood cultures were established. We now routinely use whole blood culture together with PCR and detection of HIV specific IgG, IgA, and IgM for paediatric diagnosis of HIV, all of which can be conducted with as little as 2 ml of blood.

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